Remarks

Sequence Compliance

The Office Action on page 3 objected to the sequence listing for failing to list sequences presented in a drawing. Applicants submit with this paper an updated Sequence Listing to address the basis of this objection.

Rejection under 35 USC § 103(a)

A. Claims 23-26, 28-30, and 33-35 were rejected as allegedly being obvious as set forth on page 4 of the Office Action.

Applicants have previously addressed the cited references for their lack of description or suggestion to perform PCR on a genomic sample from a subject. As previously discussed, Pasqual concerns genomic PCR on pooled thymic genomic DNA, not genomic DNA from a sample from an individual. Similarly, Arstila and Barnes have previously been discussed as having significantly higher copies of the region to be amplified present, such that one of skill in the art, knowing the low copy numbers of the rearrangements assayed in the T-cell Receptor alpha/delta (TCRAD) locus, would have thought the claimed invention impossible. GenBank GI:21363121 simply teaches a particular nucleic acid sequence. The Office Action now relies on Kolmodin as evidence that only nanogram quantities of human genomic DNA are required for amplifying a large segment of DNA for PCR.

Applicants submit that the Examiner has failed to consider the totality of Applicants' rebuttal evidence regarding the alleged obviousness over the cited references. *In re Kumar*, 418 F.3d 1335, 1338 (Fed. Cir. 2002). More particularly, Applicants submit that the Office Action of February 23, 2011, dismissed the declaration of Dr. Pasqual without providing sufficient evidence to refute Dr. Pasqual's statement. The Federal Circuit has recently mandated that the Patent Office cannot summarily dismiss a declaration without providing evidence to support the rejection. *In re Kao*, 98 USPQ2d 1799, 1807 (Fed. Cir. May 13, 2011). The Office Action of February 23, 2011 provided no evidence to counter Dr. Pasqual's statements with regard to low copy number of the target region to be amplified. The Office Action instead relied on the previously cited references as well as that of Kolmodin, none of which present evidence concerning low

copy number of the target region to be amplified and accordingly, none of which present evidence to contradict Dr. Pasqual.

Applicants would like to stress that a low starting quantity of genomic DNA is not the same as a low copy number within a low quantity of genomic DNA. All references cited concerning alleged low quantities of genomic DNA have molar equivalent amounts of the region to be amplified. The claimed invention provides the superior ability to amplify a low copy number of a TCRAD rearrangement in genomic DNA with sufficient success that the amplified product can be visualized in a gel. MPEP 716(a)(II). Such superior ability is not present in the cited references. For example, Kolmodin concerns PCR of single product that is present in two copies within each processed nucleus. Namely, the segment being amplified in Kolmodin is present twice in each cell processed for analysis. As such, each processed nucleus provides two templates for amplification, therefore providing a significant copy number of the template in the starting material. Such a copy number is not available in the same quantity of starting material in the claimed invention and, as such, Kolmodin cannot be simply extrapolated to the claimed invention.

The claimed invention is directed to visually identifying many rearrangements within the genome on an individual. The rearrangements can be particular to a single cell. As explained on page 6, lines 18-21 of the specification, the human TCRAD locus comprises consists of 54 Vα genes belonging to 41 families and 8 pseudogenes, 61 Jα genes including 3 J pseudogenes, and a single Cα gene, which leads approximately 2000 possible products in the claimed PCR, each with a low initial copy number. Each potential product to be amplified further is not within a single segment of the genome, but instead is a product of individual rearrangement within the genome of each cell. As stated in the previously filed Declaration of Dr. Pasqual that was entered with the response of January 11, 2011, due to this low copy number of rearrangements to be amplified by the PCR, one of skill in the art would expect much more starting material to be required in order to obtain a detectable product, let alone a product detectable in the gel. Similarly, as discussed in the response entered January 11, 2011, Arstila and Barnes also teach amplification of a product with a higher copy number that that available in the starting material used in the claimed invention. Due to low concentration of the

rearrangement products to be amplified, one of skill in the art would have no expectation of success.

Applicants submit with this response a further Declaration by Dr. Pasqual that highlights how Kolmodin does not overcome the deficiencies of the other cited references to render the claimed invention obvious. In particular, Dr. Pasqual distinguishes his earlier cited work from the claimed invention and describes the state of the art at the time of the present invention. Dr. Pasqual's attached declaration describes in significant detail the highest and lowest concentration of rearranged product that can statistically be present within one copy of genomic DNA. As explained, the most frequent rearrangement occurs once out of every 615 rearrangements. One of skill in the art would therefore presume that Kolmodin would require 615 times more starting material to detect even the most common rearrangement.

Moreover, as Dr. Pasqual explains in the attached declaration, one of skill in the art would appreciate this to be an over-estimation of actual rearrangements that occur. Accordingly, as Dr. Pasqual demonstrates, almost 23 μg of starting DNA are required to have sufficient copies to match Kolmodin's starting copy number. As set forth in Dr. Pasqual's previous declaration, such a quantity of genomic DNA negatively affects PCR. Accordingly, the steps of the claimed invention would not work with such a high amount of DNA.

Further, as discussed previously, Pasqual teaches away from the claimed invention, as Pasqual reports sensitivity issues based on an analysis of V-J rearrangements. One of skill in the art reading Pasqual would therefore determine that a less sensitive visualization on a more complicated set of rearrangements is not possible. As discussed above, Kolmodin does not cure these deficiencies.

Arstila and Barnes do not remedy the deficiencies of Pasqual and Kolmodin either. Arstila discloses a method for estimating the human T cell receptor (TCR) diversity, using first reverse transcriptase to increase copy number of a small fragment and then performing PCR on those templates. Accordingly, as with Kolmodin, Arstila utilizes a larger starting number of copies of the segment to be amplified than can possibly be present in the claimed invention. Similarly, Barnes discloses amplification from a much smaller in length starting material, and taking into account the mass of DNA

used, inherently Barnes, as with Arstila and Kolmodin, utilizes a much larger starting copy number of the segment to be amplified.

The Office Action acknowledges on page 4 that Wu is applicable only to claims 25 and 26. Wu concerns primer design. Wu does not teach a method for successfully amplifying a low-copy number TCRAD rearrangement in a sample from a single individual and successfully visualizing the amplified product in a gel. Accordingly, Wu does not overcome the deficiencies of Pasqual, Arstila, GenBank GI: 21363121, and Barnes that are discussed above.

Therefore, no cited reference teaches or suggests that amplification of a low-copy rearrangement can be successfully performed. All cited references teach a significantly higher amount of starting copy number of the region to be amplified. One of skill in the art at the time of the present invention would not have attempted or thought possible the claimed invention for two significant reasons: 1) the low copy number of each TCRAD rearrangement in a sample of DNA from a single human; and, 2) the amount of sample needed to obtain the copy number that those skilled in the art considered requisite. The claimed invention concerns assaying rearrangements in a single human. As a sample from a human inherently needs to be small, the copy number of the region to be amplified would be pre-determined to be too small to consider proceeding with any of the further steps of the claimed invention. Non-obviousness exists when a person of ordinary skill in the art would not have reasonably predicted the claimed invention based on the prior art and the resulting invention would not have been expected. Fed. Reg. 75: 53643-53660, 53658 (2010). As no combination of the cited references teaches that a low-copy number rearrangement in a single sample can be successfully amplified and then visualized in a gel, the claimed invention is non-obvious. Withdrawal of this rejection is requested.

B. Claim 27 was rejected as allegedly being obvious under 35 U.S.C. 103 as set forth on page 11 of the Office Action.

The deficiencies of Pasqual, GenBank GI:21363121, Arstila, Kolmodin, Barnes and Wu are discussed above. GenBank GI:21536269 teaches a particular nucleic acid sequence. Claim 27 is dependent on claim 23, and GenBank GI:21363121 does not remedy the deficiencies of the combination of Pasqual, Arstila, and Barnes to render

claim 23 obvious as GenBank GI:21536269 does not teach successful amplification of a low copy number rearrangement in the TCRAD locus in genomic DNA and successful visualization in a gel. Accordingly, claim 27 is non-obvious over the cited references. Withdrawal of this rejection is requested.

C. Claims 31 and 32 were rejected as allegedly being obvious as set forth on page 13 of the Office Action.

Claims 31 and 32 both pertain to methods comprising a step of performing the method of claim 24. Claim 24 is not obvious over the combination of previously mentioned cited references for the reasons discussed above. The only additional teaching of Dau identified by the Examiner is the comparison of the T cell repertoire of a subject to that of a healthy human subject. Dau, therefore, does not remedy the deficiencies identified above as Dau does not teach successful amplification of a low copy number rearrangement in the TCRAD locus in genomic DNA and successful visualization in a gel. Accordingly, claims 31 and 32 are not obvious over the cited references. Withdrawal of this rejection is requested.

Conclusion

The foregoing amendments and remarks are being made to place the application in condition for allowance. Applicants respectfully request entry of the amendments, reconsideration and the timely allowance of the pending claims. A favorable action is awaited. Should the Examiner find that an interview would be helpful to further prosecution of this application, she is invited to telephone the undersigned at their convenience.

Dated: **June 23, 2011**
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Respectfully submitted,
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